a probable different effect due to a different chemical structure of the two tested compounds. While the safety of squalamine has been demonstrated in clinical studies,\textsuperscript{4} no in vitro or in vivo toxicity studies have been reported for synthesized aminosterols. Nevertheless, since ASDs were reported to act as membrane disruptors, such a wide antimicrobial spectrum may encompass a potential toxicity that should be further investigated. Overall, we have demonstrated herein that ASDs possess an interesting in vitro antifungal activity,\textsuperscript{1} advocating their development for local administration, for example as aerosols especially in the context of CF. Further studies are warranted in order to evaluate their in vivo antimicrobial activities in animal models.

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Transparency declarations
None to declare.

References

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Potential synergistic activity of antifungal substances in combination with human platelets against Aspergillus fumigatus

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Keywords: Aspergillus spp., hyphal damage, antifungics, hyphal elongation

Sir,

Invasive aspergillosis is increasingly recognized in immunocompromised hosts.\textsuperscript{1} Patients with prolonged and deep granulocytopenia following chemotherapy for haematological disorders or allogeic bone marrow transplant recipients are particularly at risk.\textsuperscript{1}

Platelets are second in abundance to red blood cells in the circulation, with 1.5–4.0×10\textsuperscript{9} platelets/mL of blood in healthy individuals.\textsuperscript{2} They are essential components in haemostasis, but they also play an important role in antimicrobial host defence.\textsuperscript{3} Recently, we observed that platelets released serotonin after contact with aspergilli.\textsuperscript{3} Furthermore, platelets attenuated the virulence of Aspergillus spp., as the cell wall compound galactomannan was significantly decreased.\textsuperscript{4} Several in vitro studies have indicated that immune effector cells in combination with antifungals exert synergistic effects on aspergilli.\textsuperscript{6} However, no data are available on the effects of platelets and antifungics on aspergilli.

Two clinical Aspergillus fumigatus isolates and four antifungal agents were studied: amphotericin B (Sigma–Aldrich, Vienna, Austria); voriconazole (kindly provided by Pfizer, Vienna, Austria); posaconazole (kindly provided by Schering-Plough Research Institute, Kenilworth, NJ, USA); and caspofungin (MSD, Vienna, Austria). The MICs of all drugs used in this study were 0.5 mg/L for both isolates. Based on the MICs, drug concentrations of 0.5 and 1 mg/L were used for the platelet/drug studies. Fresh platelets were provided from the local Department of Immunology and Blood Transfusion. Platelets were collected from healthy donors by thrombopheresis using an Amicus cell separator (Baxter, Vienna, Austria). One hundred microlitres each of platelets (1×10\textsuperscript{12}/mL) and conidia (1×10\textsuperscript{9}/mL) in an effector/target ratio of 100:1 and specific concentrations of antifungals (0.5 and 1 mg/L) were inoculated into microwell plates (Greiner, Vienna, Austria) and incubated at 37°C.

The morphology of the A. fumigatus strains treated either with the combination of platelets plus antifungals or with each substance or platelets alone was investigated by assessing the germination rate and hyphal elongation, as described previously.\textsuperscript{3} Hyphal damage was assessed by a colorimetric assay with the dye 2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxynilide sodium salt (XTT; Sigma–Aldrich) plus 40 μg/mL coenzyme Q (Sigma–Aldrich) and antifungal activity was calculated as the percentage of hyphal damage.\textsuperscript{4} Each experiment was performed with platelets from one donor using duplicate or quadruplicate wells for each condition and strain, and repeated six times. Differences between mean values of both strains were statistically evaluated by repeated-measures analysis of variance followed by Dunnett’s correction for multiple comparisons. A two-sided P value of <0.05 indicated statistical significance.
Figure 1. Effect of platelets alone, antimycotics alone and the combination of antimycotics plus platelets on the germination rate of *A. fumigatus*. (a) Amphotericin B (AMB); (b) caspofungin (CAS); (c) voriconazole (VRC); and (d) posaconazole (POS). *A. fumigatus* (*n* = 2) was untreated (black bars), platelet (Pl) treated (diagonally striped bars), antimycotic treated at either 0.5 or 1 mg/L (horizontally and vertically striped bars, respectively) and combination treated with platelets plus antimycotic at either 0.5 mg/L (dotted bars) or 1 mg/L (diagonally striped bars) for 16 h at 37°C to assess inhibition of the germination rate. All experiments were repeated at least three times. The data presented are the means of six replicates for both strains. An asterisk indicates a significant difference of *P* < 0.05 compared with the untreated control (black bar). The combination of amphotericin B (0.5 and 1 mg/L) plus platelets led to a significantly lower germination rate than either single component alone.
Human platelets plus amphotericin B achieved significantly greater inhibition of the germination rate ($P<0.05$) than did amphotericin B or platelets alone (Figure 1a). The effect was additive. These results were found with both tested amphotericin B concentrations, although 1 mg/L revealed a better inhibitory effect than the lower concentration (Figure 1a). With caspofungin, the germination rate was not significantly reduced (Figure 1b). When azoles were used in combination with platelets, the inhibitory effect was found to be additive in comparison with either azole or platelets alone (Figure 1c and d). As found for the germination rate, human platelets plus amphotericin B achieved significantly greater inhibition of hyphal elongation ($P<0.05$) than amphotericin B or platelets alone. Hyphal elongation was significantly reduced in all tested aspergilli, either under platelet or caspofungin treatment. When used in combination, the effect found was to be additive. The combination of platelets plus either azole at any concentration tested did not significantly enhance the reduction of hyphal elongation. Platelets decreased the ability of hyphae to reduce XTT; however, the combination of platelets plus antymycotics had no additive effect on hyphal damage (data not shown).

Our findings indicate that platelets in combination with antimycotics exert additive effects in reducing the germination rate and hyphal elongation of *A. fumigatus* in vitro. Among the tested antimycotic substances, amphotericin B revealed the best results in combination with human platelets. However, platelets plus antymycotics were not additive for hyphal damage.

In the immunocompromised patient, inhaled *Aspergillus conidia* germinate into hyphae,1 the growing and invading structures of filamentous fungi. Consequently, blocking fungal germination and delaying hyphal growth is crucial in preventing invasive disease. In our study, the combination of platelets plus amphotericin B synergistically enhanced the antifungal activity ($P<0.05$) in reducing germination rate and hyphal elongation. Amphotericin B is known to complex with sterols in the fungal cell membrane,4 leading to pore formation and increased cell membrane permeability. This could support the antifungal activity of human platelets, by enabling the penetration of antifungal platelet factors.2

A clinical study showed that patients with invasive fungal disease had a significantly longer duration of thrombocytopenia compared with those without infection,6 suggesting that the low platelet count is related to the infection. Our *in vitro* data suggest that a normal platelet count contributes to overcome fungal infections and that platelets are capable of enhancing the efficacy of antymycotics.

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**Should tigecycline be considered for urinary tract infections? A pharmacokinetic re-evaluation**

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**Keywords:** urinary tract infections, tetracyclines, pharmacokinetics, multidrug resistance

**Sir,**

Recent debate over the use of tigecycline for urinary tract infection (UTI) treatment caused by multidrug-resistant (MDR) bacteria is based on concern of inadequate urine concentrations. First, a report of tigecycline effectiveness in a patient with recurrent UTI due to a presumed extended-spectrum *β*-lactamase (ESBL)-producing *Escherichia coli* isolate was published.1 The patient failed treatment with meropenem but the isolate from urine was reported susceptible to imipenem. The UTI was complicated by sepsis syndrome, renal abscesses, bilateral ureter catheters and possible pneumonia.1 A letter urging caution with the use of tigecycline for UTI treatment followed and summarized that only 15%–22% of tigecycline is excreted unchanged in urine.2 Although actual urine concentrations were not evaluated, the effect of renal impairment on tigecycline urinary excretion was discussed. The author asserted that ‘a 100 mg loading dose followed by 50 mg every 12 h does not ensure the ability to reach 1 to 2 mg/L in urine in a critically ill patient with chronic renal impairment’.2 To our knowledge, urinary concentration data have not been reported in patients with renal failure beyond

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